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Dissecting the SPAG6 domain that mediates interaction with Snapin

Shuo Yuan^{1,2}, Yi Tian Yap², Cassidy Wood², Qian Huang^{1,2}, Ling Zhang¹, Zhibing Zhang^{2,3}, Yunhao Liu^{1,*}

¹Department of Occupational and Environmental Health, School of Public Health, Wuhan University of Science and Technology, Wuhan, Hubei 430065, China

²Department of Physiology, Wayne State University, Detroit, MI 48201, United States

³Department of Obstetrics/Gynecology, Wayne State University, Detroit, MI 48201, United States

Abstract

Mammalian SPAG6 is located in the axoneme of central apparatus and is crucial for sperm motility and normal spermatogenesis. SPAG6 has eight conserved armadillo repeats that mediate protein-protein interaction, and the previous study showed SPAG6 interacts with C-terminus of SNARE-associated protein Snapin. To examine the SPAG6 domain that interacts with Snapin, we split the SPAG6 coding region into five segments and generated yeast expression constructs. The five SPAG6 protein fragments were successfully expressed in yeast and yeast two-hybrid assays showed that SPAG6 interacts with Snapin through the third fragment encompassing two continuous armadillo repeats. These results suggest that amino acid sequences from 199 to 280 of SPAG6 mediate the interaction between SPAG6 and Snapin. The study established a platform to further dissect the structural base for SPAG6 to bind other partners.

Keywords

SPAG6; Snapin; protein interaction

Mammalian sperm-associated antigen 6 (SPAG6) is the orthologue of *Chlamydomonas reinhardtii* PF16, which encodes a component of the central apparatus of the '9 + 2' axoneme and controls ciliary/flagellar motility. *Spag6*-deficient mice showed reduced sperm motility (Sapiro et al., 2002), and our recent studies demonstrated that it also controls spermatogenesis (Liu et al., 2019). In mouse testis, SPAG6 is present as cytoplasmic vesicles in spermatocytes, in the acrosome of round and elongating spermatids, the manchette of elongating spermatids, and central apparatus of sperm flagella (Liu et al., 2019). Full-length SPAG6 has eight conserved armadillo repeats (Fig. 1A) that mediate protein-protein interaction (Tewari et al., 2010). Using the conserved domain as bait for a yeast two-hybrid screen, multiple binding partners were identified, including Snapin (Liu et al., 2019). The dynamic localization and the existence of multiple binding partners suggest that SPAG6 is a multiple functional protein, and it might play different roles through binding to different

*Correspondence: yunhaoliu@wust.edu.cn.

proteins during different spermatogenesis phases. Snapin is predicted to have the highest binding affinity as evaluated by appearance frequency in the yeast two-hybrid screen, and SPAG6 binds to the C-terminus of Snapin (Liu et al., 2019). In order to dissect the SPAG6 domain that mediates interaction with Snapin, *Spag6* coding region was amplified as 5 continuous fragments (Fig. 1B), with nucleotides from 1 to 339, 340 to 591, 592 to 870, 871 to 1170, and 1171 to 1521 respectively, and “1” is the first coding nucleotide (A). The five fragments were cloned into NdeI/BamHI sites of pGBK-T7. The five constructs were co-transformed into competent AH109 yeast with Snapin/pGAD-T7. The co-transformed yeast was grown on the plates with non-selective medium (complete drop-out medium lacking tryptophan and leucine) or selective medium (lacking tryptophan, leucine, and histidine). All yeast grew on the plates with non-selective medium (Fig. 1C, upper); however, only the yeast co-transformed with fragment 3 and positive control grew on the plate with selective medium (Fig 1C, lower). To examine if the proteins were expressed in the yeast, Western blot was conducted using lysates from the yeast grown from the non-selective medium. All five fragments expressed proteins as expected sizes (Fig. 1D). These results revealed that SPAG6 interacts with Snapin through the third fragment, the amino acid from 199 to 280. This study not only dissects the SPAG6 domain that mediates interaction with Snapin, but also establishes a platform to further investigate how SPAG6 interacts with other proteins in the regulation of spermatogenesis.

Acknowledgments

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(A)

1 MSQRQVLQVFEQYQKARTQFVQMVAELATRPQNIETLQAGVMSLLRPLL
 51 LDVVPTIQQTAALALGRLANYNDLAEAVVKGDIPLQVLSLAEQNRIFYK
 101 KAAAFVLRVAGKHSPQLAQAIIVDCGALDTLVICLEDFFDGPVKEAAAWALG
 151 YIARHNTELSQAVVDAGAIPLLVLCIQEPEIALKRIAASALS DISKHSPE
 201 LAQTVDVDAIAHLAQMLNPDALKRQVLSALSQIAKHSVDLAEMVVEA
 251 EIFPVVLTCLKDKDEYVKKNACTLIREIAKHTPELSQLIVNAGGVAAVID
 301 CIGSCKGNIRLPGIMMLGYVAHSENAMAVIISKGVPLSICLSEEPED
 351 HIKAAAWALGQLGRHTPEHARAVAVTNTLPVLLSLYMSPESSDLQLKS
 401 KKAIAKNILQKCTYLPALPEFLYDAPPNILKHVVGGQFSKVLPHDSKARRLF
 451 VTSGGLKKVQEIKAEPGSLLEQYINSINNCYPPEIVRYSPGYSDTLQLQR
 501 VDSYQPL

(B)

(C)

(D)

Figure 1.

(A) Analysis of mouse SPAG6 by UniProtKB showed it contains eight conserved armadillo repeats (ARMs) that are underlined and numbered. (B) Schematic representation of SPAG6 amino acid sequences and eight ARM domains, and the location of primers used to amplify five fragments of SPAG6. (C) Interaction between Snapin and five fragments of SPAG6 (SPAG6-F1~F5) in yeast. Yeast AH109 was transformed with the indicated plasmids and then grew on non-selective media (SD/-Leu/-Trp) and selective media (SD/-Leu/-Trp/-His), respectively. Only the fragment 3 of SPAG6 interacted with Snapin. (D) Expression of five fragments of SPAG6 and Snapin in yeast. Western blotting confirmed the protein expression in yeast lysates using indicated antibodies (c-Myc tag and HA tag are present in the vector pGBK-T7 and pGAD-T7 respectively, and fused to the N-terminus of SPAG6 or Snapin).